

CLAIMS

- 1) An artificial promoter characterized for being a recombinant DNA molecule promoting expression in plant cells of any DNA sequence fused to its 3' end, comprising:
 - a) A 5' transcription regulator element followed by,
- 5) b) An artificial core promoter comprising a TATA box, a nucleotide sequence with a GC content lower than 64% and a transcription initiation site fused in its 3' end to,
- c) A synthetic nucleotide sequence transcriptable but not translatable, conformed by a first chimerical Exon, an artificial Intron able to enhance the expression of genes fused to it in plant cells, and a second chimerical Exon with translation enhancement properties of any 10 gene inserted downstream.
- 2) An artificial promoter according to Claim 1 characterized for being a recombinant DNA molecule where the 5' transcription regulation element is artificial.
- 3) An artificial promoter according to Claim 1 characterized for being a recombinant DNA molecule where the 5' transcription regulation element is homologous to a DNA sequence 15 that naturally enhances and/or regulates gene expression in plant cells.
- 4) An artificial promoter according to Claim 3 which 5' transcription regulation element comes from rice actin-1 gene.
- 5) An artificial promoter according to Claim 4, where its 5' transcription regulation element comprises the region from -43 to -310 from rice actin-1 gene transcription 20 initiation site.
- 6) An artificial promoter according to Claim 5 wherein the 5' transcription regulation element nucleotide sequence corresponds to sequence in SEQ ID NO: 10 or to a fragment of it.
- 7) An artificial promoter according to Claim 5 wherein the 5' transcription regulation 25 element nucleotide sequence corresponds to sequence in SEQ ID NO: 11 or to a fragment of it.
- 8) An artificial promoter according to Claim 3 where its 5' transcription regulation element comes from maize ubiquitine-1 gene.
- 9) An artificial promoter according to Claim 8 wherein the nucleotide sequence comprises 30 the region from -299 to -855 from maize ubiquitine-1 gene transcription initiation site.
- 10) An artificial promoter according to Claim 9 wherein the 5' transcription regulation element corresponds to sequence in SEQ ID NO: 19 or to a fragment of it.
- 11) An artificial promoter according to Claims 2 and 3 where its 5' transcription regulation element is an *as-1*-like transcriptional enhancer.

- 12) An artificial promoter according to Claim 11 where nucleotide sequence of as-1-like transcriptional enhancer is essentially identical to the sequence fragment corresponding to nucleotides 7 to 26 in SEQ ID NO: 13, or to the complementary sequence.
- 13) An artificial promoter according to Claim 3 where its 5' transcription regulation element comes from a viral promoter.
- 5 14) An artificial promoter according to Claim 13 where its 5' transcription regulation element comes from CaMV 35S promoter.
- 15) An artificial promoter according to Claims 2 and 3 wherein the 5' transcription regulation element controls gene expression in plant cells with development-, organ- or tissue-specificity.
- 10 16) An artificial promoter according to Claim 15 where its 5' transcription regulation element controls expression in seeds.
- 17) An artificial promoter according to Claim 16 where its 5' transcription regulation element comes from rice gluteline B-1 gene.
- 15 18) An artificial promoter according to Claim 17, where its 5' transcription regulation element comprises a fragment of the region from -31 to -245 from the rice gluteline B-1 gene transcription initiation site.
- 19) An artificial promoter according to Claim 18 wherein the 5' transcription regulation element corresponds to sequence in SEQ ID NO: 21 or to a fragment of it.
- 20 20) An artificial promoter according to Claim 15 wherein the 5' transcription regulation element controls gene expression in plant cells under biotic or abiotic stress.
- 21) An artificial promoter according to Claim 15 where its 5' transcription regulation element controls gene expression in wounded plant tissues.
- 25 22) An artificial promoter according to Claim 1 wherein the 5' transcription regulation region comprises 2 or more regulator elements from different origin operatively fused, which individually responds to any of the characteristics said on claims from 2 to 21.
- 23) An artificial promoter according to Claim 1 wherein the first Exon from the artificial Exon/Intron/Exon region comprises sequence motifs C and A rich.
- 24) An artificial promoter according to Claim 1 wherein the first Exon from the artificial Exon/Intron/Exon region comprises sequences wherein are frequently repeated the motif CTCC and/or its homologous sequences CTC, TCC and TC.
- 30 25) An artificial promoter according to Claim 1, which Intron from the artificial Exon/Intron/Exon region comprises sequences where the CTCC motif and/or its homologous sequences CTC, TCC and TC are frequently repeated.

26) An artificial promoter according to Claims from 23 to 25 wherein nucleotide sequence of the artificial Exon/Intron/Exon region corresponds to SEQ ID NO: 6 or to a fragment of it.

27) An artificial promoter according to Claim 1 wherein the second Exon from the 5 artificial Exon/Intron/Exon region comprises sequence motifs with high C and A content.

28) An artificial promoter according to Claim 1 wherein the second Exon from the artificial Exon/Intron/Exon region comprises at least a sequence 83 % homolog with motif HCAYYY (H= C ó T ó A; Y= C ó T).

29) An artificial promoter according to Claims 27 and 28 wherein nucleotide sequence of 10 the second Exon from the artificial Exon/Intron/Exon region corresponds to sequence in SEQ ID NO: 1.

30) An artificial promoter according to Claims from 23 to 29 wherein nucleotide sequence of the artificial Exon/Intron/Exon region corresponds to SEQ ID NO: 8 or with a fragment of it.

15 31) An artificial promoter according to any of the claims from 1 to 30, wherein nucleotide sequence of the artificial Exon/Intron/Exon region corresponds, at least partially, to sequence in SEQ ID NO: 20.

32) A DNA fragment from an artificial promoter according to Claims from 1 to 31 such that, when fused to any promoter functional in plants, contributes to enhance expression of 20 DNA sequences controlled by said promoter.

33) An artificial promoter fragment according to Claim 32 able to enhance translation of genes fused to it.

34) An artificial promoter fragment according to Claim 33, comprising at least a sequence 83 % homolog with motif HCAYYY (H= C ó T ó A; Y= C ó T).

25 35) An artificial promoter fragment according to Claim 33 with sequence motifs C and A rich.

36) An artificial promoter fragment according to Claim 33 wherein the nucleotide sequence corresponds to that on SEQ ID NO: 1.

37) An artificial promoter fragment according to claims from 33 to 36 that contributes to 30 enhance translation of mRNA's produced from the CaMV 35S promoter in plant cells.

38) An artificial promoter fragment according to Claim 32 corresponding to an Exon/Intron/Exon region.

39) An artificial promoter fragment according to Claim 38 wherein the first Exon comprises sequence motifs C and A rich.

- 40) An artificial promoter fragment according to Claim 38 wherein its first Exon comprises sequences where are frequently repeated the motif CTCC and/or its homologous sequences CTC, TCC and TC.
- 41) An artificial promoter fragment according to Claim 38 wherein its Intron comprises sequences where are frequently repeated the motif CTCC and/or its homologous sequences CTC, TCC and TC.
- 42) An artificial promoter fragment according to Claim 38 wherein nucleotide sequence corresponds with sequence in SEQ ID NO: 6.
- 43) An artificial promoter fragment according to Claim 38 wherein nucleotide sequence corresponds with sequence in SEQ ID NO: 8.
- 44) An artificial promoter fragment from claims 38 to 43 that contributes to enhance the expression of any gene under the control of CaMV 35S promoter in plant cells.
- 45) An artificial promoter fragment according to Claim 32 corresponding to an as-1-like transcriptional enhancer.
- 46) An artificial promoter fragment which nucleotide sequence is essentially identical to that of the fragment corresponding to nucleotides 7 to 26 in SEQ ID NO: 13, or its complementary sequence.
- 47) An artificial promoter fragment according to Claim 32 corresponding to a 5' transcription regulation element.
- 48) An artificial promoter fragment according to Claim 47 wherein the 5' transcription regulation element comes from rice actin-1 gene.
- 49) An artificial promoter fragment according to Claim 48 wherein nucleotide sequence comprises a fragment from -43 to -310 from rice actin-1 gene transcription initiation site.
- 50) An artificial promoter fragment according to Claim 49 wherein nucleotide sequence corresponds to sequence in SEQ ID NO: 10 or to a fragment of it.
- 51) An artificial promoter fragment according to Claim 49 wherein nucleotide sequence corresponds to sequence SEQ ID NO: 11 or to a fragment of it.
- 52) An artificial promoter fragment according to Claim 47 where its 5' transcription regulation element comes from maize ubiquitine-1 gene.
- 53) An artificial promoter fragment according to Claim 52 wherein nucleotide sequence comprises the region from -299 to -855 from maize ubiquitine-1 gene transcription start site.
- 54) An artificial promoter fragment according to Claim 53 wherein the 5' transcription regulation element corresponds to sequence in SEQ ID NO: 19 or to a fragment of it.

55) A cassette for the expression of DNA sequences in plant cells containing an artificial promoter responding to any of the claims from 1 to 31.

56) A cassette for the expression of DNA sequences in plant cells containing a transcription enhancer element functionally fused to a DNA fragment responding to any of the claims from 32 to 54.

57) A DNA vector for plant cell transformation comprising one of the expression cassettes characterized in claims 55 or 56.

58) A bacterial cell carrying vector on claim 57 and its descendants.

59) A plant cell transformed with vector on claim 57, and its descendants.

10 60) A plant cell according to Claim 59 expressing the DNA fragment under the control of the artificial promoter in the expression cassette introduced by the means of the vector described on claim 57.

61) A plant cell according to Claim 59 with the expression cassette characterized on claim 55 or 56 stably integrated into its genome.

15 62) A transgenic plant regenerated from the plant cell characterized on claim 61.

63) A transgenic plant according to Claim 62 expressing the DNA fragment under the control of the artificial promoter comprised into the expression cassette introduced by the means of the vector described on claim 57.

64) Transgenic plant descendants characterized in claim 63.

20 65) Plants according to Claim 64 being dicots.

66) Plants according to Claim 65 being Solanaceae.

67) Plants according to Claim 66 belonging to one of the following species: tobacco, tomato or potato.

68) Plants according to Claim 64 being monocots.

25 69) Plants according to Claim 68 being gramineae.

70) Plants according to Claim 69 belonging to one of the following species: rice, sugarcane, maize, wheat or barley.

71) The purification or use of recombinant proteins produced by cells or plants according to Claims 60 or 63 as a result of the expression of the DNA fragments sited under the control of the artificial promoter comprised into the expression cassette introduced by the means of the vector described on claim 57.